New and Notable

Revealing the Cellular Basis of Heart Failure

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''Nature loves to conceal herself''
—Heraclitus, ''On Nature''

It is a sobering fact that 25 centuries after the first recorded description of heart failure we are just beginning to understand the cellular and biophysical changes that underlie this condition. Clearly, physiology does not reveal herself easily. Roughly 2500 years ago Hippocrates was able to give a detailed account of the signs and symptoms of heart failure (HF) including the peripheral edema that accompanies this pathology (1). Currently physiologists and clinicians rely on a definition of heart failure of the type proposed by Eugene Braunwald (2) as ''a pathophysiological state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirements of the metabolizing tissues''. What therefore are the cellular mechanisms that lead to this condition?

Different types of heart failure have been described and these depend on which portion of the contraction-relaxation cycle (duty cycle) is affected. Systolic HF is often accompanied by a permanent reduction in sarcoplasmic reticular (SR) Ca content. How and why this decline takes place is extremely important as it underlies the loss in contractility and therefore cardiac pump function that defines HF. Using a pacing-induced canine model of HF, Belevych et al. (3) report some results in this edition of the Biophysical Journal that go far toward answering these questions. It is particularly notable that these authors are able to provide data that suggest that decreased SR Ca content in HF is due to a single cause: an increased SR Ca leak. This is a significant departure from other explanations, which, for example, suggest that it is due to impaired SERCA 2 activity or increased Na-Ca exchanger (NCX) activity, e.g., Schwinger et al. (4) and Hasenfuss et al. (5). In addition, the authors suggest that the leak could contribute to the slowed kinetics of the cytosolic Ca transients in HF myocytes. This is usually explained by reduced SERCA or increased NCX activity. Before discussing their data, a brief mention of some key aspects of cardiac excitation-contraction coupling may be helpful.

The SR is an intracellular store of Ca that is periodically released to activate contraction. Release takes place through clusters of large Ca channels or Ryanodine receptors (RyRs) in the SR membrane. Release is activated when small quantities of Ca pass through voltage-gated Ca channels in the sarcolemma, which in turn gate the RyRs to which they are closely apposed. When RyRs are gated they release much larger quantities of Ca from the SR. Ca that initially entered the cell and gated the RyRs during each duty cycle is extruded by the NCX. Released Ca is resequestered by an SR membrane bound Ca pump. It is important to appreciate that during steady-state contractions two conditions must hold. The first is that during each duty cycle the total SR Ca released must equal the subsequent Ca uptake by the SR. Second, total Ca efflux must balance total Ca influx across the cell membrane within the duty cycle.

The difficulty in explaining why SR Ca content is reduced in heart failure is partly due to the difficulty of explaining mechanisms that can overcome powerful homeostatic processes proposed by Eisner et al. (6) that maintain a stable SR content, but nevertheless leave some regulatory capacity. However, we shall see that the proposals of Eisner et al. (6) are consistent with a new setpoint for the SR content. Moreover, because excitation-contraction coupling is complex, difficult measurements are required to dissect the cellular mechanisms that underlie HF. To appreciate how SR Ca content can be reduced it is helpful to consider the following equations together with some assumptions that, as we shall see, Belevych et al. (3) completely justify with their findings. Here we derive simple equations that relate release flux to parameters associated with SR functions that are likely to control it. The Ca release flux through unit area of the SR is given by the equation

$$J_{SR} = nPo\,P_{RyR}[Ca]_{SR}. \qquad (1)$$

J_{SR} is the flux through unit area of the SR membrane, n is the number of RyRs, Po is the open probability of a single RyR, P_{RyR} is the permeability coefficient for the flux through a single RyR, and $[Ca]_{SR}$ is the SR Ca concentration. The term $P_{RyR}\,[Ca]_{SR}$ is the unitary flux through a single RyR and it produces a unitary current i_{RyR}. We assume that no voltage exists across the SR membrane.

A straightforward extension of Eq. 1 is

$$J_{SRT} = nP_{RyR}\int_{t_o}^{t_o+\Delta t} Po(t)[Ca]_{SR}(t)dt. \qquad (2)$$

Equation 2 relates total release flux during the duty cycle, J_{SRT} (the integral of J_{SR}) to RyR open probability, permeability, and the integral of $[Ca]_{SR}$. The value $t_o+\Delta t$ is the time from the beginning to end of the duty cycle. If we assume J_{SRT} declines and Po increases for all t during the duty cycle, then from Eq. 2 it must follow that $[Ca]_{SR}$ declines for all t, i.e., SR Ca content declines. However, although Eq. 2 suggests that, with the stated assumptions, SR content will decline, it does not explain the mechanism of this decline and the sequence of changes

Submitted July 22, 2007, and accepted for publication August 8, 2007.

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Editor: David A. Eisner.

0006-3495/07/12/3731/02 $2.00

doi: 10.1529/biophysj.107.116541

that might take place to produce it. In fact, we shall argue from Belevych et al. (3) that, if the primary effect of HF is to increase *Po*, then a secondary effect of this will be to reduce both release flux and SR uptake with a consequent decline in content.

Belevych et al. (3) have obtained a comprehensive set of results that are entirely consistent with this and provide a basis for explaining the mechanism that leads to reduced SR Ca content. Using elegant techniques to monitor SR Ca they have been able to show that SR Ca leak has increased in cells isolated from hearts in HF. It seems that the likely explanation for this is an increase in the value of *Po* during the duty cycle. In fact, Marx et al. (7) have suggested that PKA phosphorylation of the RyR channel dissociated the regulatory protein FKBP12.6 from the channel. Their results, although somewhat controversial, indicate an increased *Po* and increased subconductance states of RyRs that have been hyperphosphorylated. Marx et al. (7) noted that cells in HF show similar properties and, in addition, prolonged subconductance states. These could increase leak during diastole. Belevych et al. (3) also show that while SERCA 2 activity remains unchanged in HF, resequestration is slowed and presumably, within a duty cycle, the total SR Ca uptake has declined. Thus it is likely that total release has also declined to maintain a flux balance during the duty cycle. Therefore, the results from Belevych et al. (3) lead us to conclude from Eq. 2 that SR Ca content has declined, as our initial illustration suggested. Indeed these authors provide evidence that SR content is reduced in their model, consistent with the findings of others (8) and the aforementioned conclusion. We should now consider a possible sequence of events that might lead to a reduction in SR Ca content. If an initial effect of HF is to increase the *Po* of RyRs this will increase the total SR Ca released. This will, in turn, increase the Ca transient and a larger fraction of the SR Ca will be extruded by NCX. The SR will resequester less Ca, and SR Ca will decline. This will continue until SR fluxes come into balance at a lower SR Ca and hence reduced release. Thus one may view (at least in this model of HF) an increase in *Po* as the immediate cause of a change in release flux, which leads to a decline in SR Ca content. However, this will not be maintained unless transsarcolemmal fluxes are also in balance. We will deal with this issue shortly. In the presence of Ruthenium red (which inhibits RyRs), these authors show that the SR seems capable of filling close to control values. This clearly suggests that the extent to which the SR leaks Ca ultimately controls the transmembrane fluxes and the SR Ca content. This also suggests that the decline in content could proceed from a single cause: an increase in leak and therefore *Po* for the RyRs.

We indicated earlier that one of the difficulties in explaining permanent declines in SR Ca content during HF is that homeostatic mechanisms have been demonstrated to maintain SR Ca content (6). It is now known that the relationship between SR Ca content and the Ca transient (and therefore release flux) is steep (9). Thus, a relatively small decline in content can produce a significant decline in release with a reduced Ca transient. This in turn will produce a reduced efflux of Ca by NCX (as Belevych et al. (3) acknowledge). Without any change in Ca influx at the beginning of the duty cycle one expects a gain in cell and hence SR Ca. This does not occur. It seems therefore that since a flux balance must be maintained across the sarcolemma during the duty cycle any reduction in efflux during the transient must be offset by an increase of efflux after the transient is over but before the next stimulus. The increased leak of Ca during diastole is a good candidate for this. Indeed, the increase activity of NCX observed by Belevych et al. (3) may increase the efflux of Ca (initiated by increased RyR leak) during the period when Ca is low before the next stimulus. The system would still be capable of regulating SR Ca content at this new level since any further change in the flux balance would result in compensatory changes in SR Ca content. Clearly careful measurements of sarcolemmal Ca fluxes during the duty cycle are needed to clear up this ''loose end''.

It appears therefore that in a single study, using state-of-the-art methods, Belevych et al. (3) have succeeded in providing much of the necessary information to explain how SR Ca content can decline during HF without losing essential homeostatic mechanisms. The study invites, among other things further investigation into the mechanism of the leak as well as the time course with which the leak develops. We can undoubtedly look forward to this in the future.

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